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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: TE RIELE *et al.*

Title: HOMOLOGOUS RECOMBINATION IN MISMATCH REPAIR INACTIVATED EUKARYATIC CELLS

Appl. No.: 09/884,877

Filing Date: June 20, 2001

Examiner: Unassigned

Art Unit: 1633

PRELIMINARY AMENDMENT

Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Sir:

Prior to examination of the present Application, Applicants respectfully request that the application be amended as follows:

IN THE SPECIFICATION:

After the claims, please insert the Abstract that is found on the separate sheet below.

ABSTRACT

A mammalian cell having a mismatch repair-deficient phenotype is provided, where one or both alleles of a gene essential for mismatch repair, such as an *Msh* gene, are inactivated. Using this cell in a gene knock-out methodology advantageously allows efficient homologous recombination, even when the DNA sequences of the donor and recipient sequences diverge by significantly more than 0.6%.

IN THE CLAIMS:

Please cancel claims 1-23 without prejudice or disclaimer and add claims 24-49.

24. (New) A method of making a cell having a mismatch repair deficiency phenotype, comprising inactivating the mismatch repair system of the cell.

25. (New) The method of claim 24, wherein the cell is a mammalian cell.

26. (New) The method of claim 24, wherein the mismatch repair system is inactivated by disrupting both copies of a gene essential for mismatch repair.

27. (New) The method of claim 26, wherein the gene essential for mismatch repair is a mammalian *Msh2* gene or a mammalian homologue of a *mutL* gene.

28. (New) The method of claim 27, wherein the gene essential for mismatch repair is a mammalian *Msh2* gene.

29. (New) A diploid mammalian cell made by the method of claim 24.

30. (New) The cell of claim 29, wherein one *Msh2* allele is inactivated.

31. (New) The cell of claim 30, wherein the *Msh2* allele contains an insertion of a *hyg* marker between codons 588 and 589.

32. (New) The cell of claim 29, wherein both *Msh2* alleles are inactivated.

33. (New) The cell of claim 32, wherein the cell is a *dMsh2-9* cell.

34. (New) A method for stably incorporating through homologous recombination a donor DNA molecule into the genome of a mammalian recipient cell that has a mismatch repair deficiency phenotype, comprising transforming the recipient cell having a mismatch repair deficiency phenotype with a donor DNA molecule that is obtained from a donor cell, wherein the donor DNA molecule is stably integrated into the genome of the recipient cell through homologous recombination with a homologous recipient DNA molecule, and wherein the sequence of the donor DNA molecule is not identical with the sequence of the homologous recipient DNA molecule.

35. (New) The method of claim 34, wherein the nucleotide sequence of the donor DNA molecule diverges from the nucleotide sequence of the homologous DNA molecule in the recipient cell by about 0.6% to about 5%.

36. (New) The method of claim 34, wherein the nucleotide sequence of the donor DNA molecule diverges from the nucleotide sequence of the homologous DNA molecule in the recipient cell by about 0.6% to about 30% in the region where homologous recombination can take place.

37. (New) The method of claim 34, wherein the mammalian recipient cell is an embryonic stem cell or a germ line cell.

38. (New) The method of claim 34, wherein the mammalian recipient cell is obtained from a cell line that is cultured *in vitro*.

39. (New) The method of claim 34, wherein the mammalian recipient cell is obtained from an organ of a mammal.

40. (New) The method of claim 34, wherein at least one of the nucleotide base or base pairs in the donor DNA is modified *in vitro* prior to transformation.

41. (New) The method of claim 40, wherein the modification is a point mutation, an insertion of base pairs, or a deletion of base pairs from the donor DNA molecule, and wherein the modified donor DNA molecule diverges from the nucleotide sequence of the homologous DNA molecule in the recipient cell by about 0.6% to about 5%.

42. (New) The method of claim 40, wherein the modification is a point mutation, an insertion of base pairs, or a deletion of base pairs from the donor DNA molecule, and wherein the modified donor DNA molecule diverges from the nucleotide sequence of the homologous DNA molecule in the recipient cell by about 0.6% to about 30% in the region where homologous recombination can take place.

43. (New) The method of claim 34, wherein the donor DNA molecule is a chromosomal DNA fragment that is inserted into a YAC or cosmid vector.

44. (New) The method of claim 34, wherein the donor DNA molecule is a double-stranded oligonucleotide 10-100 bases in length, and wherein the nucleotide sequence of the donor DNA molecule diverges from the nucleotide sequence of the

homologous DNA molecule in the recipient cell by at least one base pair, but no more than 5% of all base pairs.

45. (New) The method of claim 34, wherein the donor DNA molecule is a single -stranded oligonucleotide 10-100 bases in length, and wherein the nucleotide sequence of the donor DNA molecule diverges from the nucleotide sequence of the homologous DNA molecule in the recipient cell by at least one base, but no more than 5% of all bases.

46. (New) The method of claim 34, wherein the donor DNA molecule comprises a selectable marker gene flanked by two sequences, wherein one flanking sequence has at least 95% sequence identity to the corresponding sequence of the recipient DNA molecule and the other flanking sequence comprises a repetitive sequence.

47. (New) The method of claim 46, wherein the repetitive sequence is a long interspersed element (LINE) or a short interspersed element (SINE).

48. (New) A method of making a transgenic animal, comprising (a) inserting a genetically modified stem cell prepared according to the method of claim 34 into a blastocoel, (b) implanting the blastocoel into a womb of a female host animal to make the female animal pregnant, and (c) carrying the pregnancy to term to obtain a viable transgenic animal.

49. (New) A transgenic animal made by the method of claim 48.

REMARKS

Applicants respectfully request that the foregoing amendments be made prior to examination of the present application.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date December 26, 2001

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SUBSTITUTE SPECIFICATION UNDER C.F.R. §1.125

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Sir:

Applicants attach a Substitute Specification with proper margins in compliance with 37 C.F.R. § 1.52, as requested in the Notice to File Missing Parts. Applicants have amended further the specification to correct typographical errors and to reorder and rewrite portions of the written description for greater clarity. The Substitute Specification does not introduce new matter. A marked up version of the written description indicating the changes also is attached. Deletions appear as overstrike text surrounded by { }, and additions appear as bold text surrounded by [ ].

REMARKS

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date December 26, 2001

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